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Evaluation of Antioxidant Activity of Aqueous and Ethanolic Extracts of *Myrianthus Holstii* (Cecropiaceae) Barks, *in Vitro*

Silue Kalamourou[1], Doumbia Idrissa[1, 2*], Nimaga Daouda[1], Yéo Sounta Oumar[1], Yapi Houphouet Felix[2], N'guessan Jean David[2], Djaman Allico Joseph[2, 3]

[1]Food and Bioproducts Processes Laboratory, Agronomic, Forestry and Environmental Engineering Department, University of Man, PO Box 20 Man, Ivory Coast
[2]Biochemical Pharmacodynamy Laboratory, Biosciences Department, Felix Houphouet-Boigny University, PO Box 582, Abidjan 22, Ivory Coast
[3]Biochemical Laboratory of Pasteur Institute of Ivory Coast, PO Box 490, Abidjan 01, Ivory Coast

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***Corresponding author:** Doumbia Idrissa

Abstract **Original Research Article**

The purpose of this study is to evaluate the antioxidant activity of *Myrianthus holstii* (Cecropiaceae), a plant used in traditional medicine against gastroenteritis in western Côte d'Ivoire (Man). The extraction yields of the ethanolic extract (EE) and the aqueous extract are respectively 6.4% and 7.95%. Determination of the total phenol content by the colorimetric method gave the following results: EAeC (27.67 ± 1.45 mg EAG / g of extracts) and EEec (428.70 ± 1.67 mg EAG / g of extracts). The flavonoid assay using the AlCl3 aluminum chlorides method gave the following results: EAec (12 ± 0.57 EQ / g extracts) and EEec (53.33 ± 3.75 EQ / g of extracts). The evaluation of the antioxidant activity of extracts was carried out according two methods: the free radical scavenging by the DPPH and the measurement of the reducing power (FRAP). The results obtained indicate that ethanolic extract contains more polyphenolic compounds than the aqueous extract. Ethanolic extract (EAec) antioxidant properties (EEec, IC50 = 03.20 ± 0.45 mg/mL) are also greater than those of the aqueous extract (EAec, IC50 = 43.30 ± 0.40 mg/mL). This antioxidant activity which remains close to that of vitamin C (01.25 ± 0.02) could represent an additional asset in the management of pathologies linked to oxidative stress.

Keywords: Antioxidant Activity, *Myrianthus Holstii*, *in Vitro*. ethanolic extract (EE).

INTRODUCTION

The development of new antioxidants with an antioxidant capacity of better quality and lower toxicity is essential to fight against oxidation phenomena. Free radicals are thought to be at the origin of certain chronic diseases (cardiovascular, cancerous and neurodegenerative diseases) as well as aging [1] This would cause cellular dysfunction and would be involved in various pathologies such as cardiovascular disease, cancer, diabetes, neurodegenerative diseases (Alzheimer's, Parkinson's) and the aging process [2].

Similarly, some synthetic antioxidants have shown a potential health risk, including a possible carcinogenic effect. It is therefore necessary to find new sources of low-risk, inexpensive and natural antioxidants for use in foods, pharmaceutical preparations and thus replace synthetic antioxidants [3]. For this reason, new sources of natural antioxidants are actively sought [4, 5].

Eyes then turn more and more to the medicinal plants supplying bioactive substances. Natural products are an important source for the search for new active compounds used to treat many diseases [6, 7]. Many studies have been carried out on the biological properties of extracts of some plants and have allowed the discovery of many active ingredients used in modern medicine for the synthesis of drugs [8]. Thus, it is estimated that approximately more than half of bioactive molecules in clinical use come from plants. They contain several varieties of antiradical molecules: phenolic compounds, nitrogen compounds, vitamins, terpenoids, which have a strong antioxidant activity [9]. Plants are therefore natural sources of antioxidant substances that are intended to protect them against stress [10].

Myrianthus holstii, a medicinal plant used the populations of western Côte d'Ivoire for the belly enema. Scientific studies have shown the leaves are traditionally used in veterinary medicine to treat

wounds on teats and swelling of the head followed by coughing of cows. Similarly, stem bark, trunk of this species are mixed with a branch for the enema and can fight against diarrhea, dysentery, cholera, fatigue, miscarriages and fortifies children [11]. It is a plant widely used in traditional medicine because of its febrifuge, depurative, antipyretic, antispasmodic, fortifying, stimulating, antidiarrheal, laxative and antimalarial properties [12]. The present research aims to verify the antioxidant activity of the aqueous and ethanolic extracts of the bark of *Myrianthus holstii.*

MATERIAL AND METHODS

Plant Material

The barks of *Myrianthus holstii* ((Cecropiaceae), harvested in october 2018 in Kassiapleu near Man (western of Côte d'Ivoire) have been identified by the National Center of Floristry at the University Felix Houphouet Boigny (Cocody-Abidjan). A specimen of the plant was deposited in the herbarium of this Center.

Preparation of Aqueous Extract

Myrianthus holstii bark powder (100g) were macerated for 48 hours in 1L of distilled water. The macerate has been wrung into a square of sterile tissue, filtered successively on cotton wool and one fold on filter paper (Whatman paper® 2mm). The filtrate was dried slowly in the stove at 50°C. The powder obtained was stored in a hermetically sealed jar and refrigerated at 4° C [13].

Preparation of Ethanolic 70% Extract

It was carried out using modified. method. A mass of 20g of plant powder was added in 100ml of ethanol 70% and subjected to maceration for 72 hours . The macerate was treated according to the same procedure like the aqueous extract [14].

Antioxidant Activity

Total Phenolic Content

The total phenolic content of the extract was determined separately using the method of [15, 16]. The calibration curve was prepared by mixing methanolic solution of gallic acid (1ml ; 0-100 g/ml) with 5ml Folin-Ciocalteu reagent and sodium carbonate (4ml, 1M). We measured absorbance at 765nm and drew the calibration curve. 1ml of extract (100 g/ml) was also mixed with the reagents above and after 15min, the absorbance was measured to determine plant total phenolic contents. Experimentations were carried out in triplicate. The total phenol values are expressed in terms of gallic acid equivalent (mg GAE/g of extract), which is a common reference compound.

Total Flavonoids Content

The total flavonoids content was analyzed by aluminum chloride method [17,18]. Each plant extract (0.5ml of 1:100µg/ml) was mixed with 1.5mL methanol, 0.1mL of $AlCl_3$ (10%), 0.1mL of 1M potassium acetate and 2.8 mL of distilled water. The mixture was allowed to stand for 30min at room temperature (25 °C) and absorbance was measured at 415nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared by preparing quercetin solutions at concentrations ranging from 0 to 100µg/ml in methanol. Total flavonoids contents were expressed as mg of Quercetin equivalents (QE)/g of extract. Samples were analyzed in triplicates.

Chelating Ability

Chelating ability of Fe^{2+} was determined according to the method of [19,16]. Fe^{2+} was monitored by measuring the formation of ferrous iron-ferrozine complex at 562 nm. Different concentration of extract (1ml) in 3.7ml of methanol was mixed with $FeCl_2$ (0.1ml, 2mM) and ferrozine (0.2ml, 5mM). The resulting mixture was shaken and left to stand for 10min at room temperature. EDTA was used as standard control. The absorbance of the resulting solution was measured at 562nm.

The capability to chelate the ferrous iron was calculated using the following equation: Chelating Effect (%) = [(Ao - A1)/ Ao] X 100;

Ao was the absorbance of the control (containing all reagents except the test compound) and A1, the absorbance in presence of sample of extract and standard.

Free Radical Scavenging Activity

Hydrogen atom or electron donating abilities of the compounds were measured from the bleaching of the purple-coloured methanol solution of 2,2-diphenyl-1-picryl hydrazyl (DPPH). This spectrophotometric assay uses the stable free radical, DPPH as a reagent [20]. Different concentrations of each extract were added, at an equal volume, to methanolic solution of DPPH (100µM). After 30 min at room temperature, the absorbance was recorded at 517 nm. Test was repeated for three times. Vitamin C was used as standard control. The DPPH radical scavenging effect was calculated as inhibition of percentage (I %) using the following formula:

I % = (A Blank-A Sample/A Blank) ; A blank is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. The values of inhibition were calculated for concentrations of the extract. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Chemicals Reagents

All chemicals used were of analytical grade. Methanol, aluminum chloride, potassium acetate, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ferrous chloride,

ferrozine, potassium ferricyanide, Folinciocalteu reagent, standards such as Lascorbic acid, ethylenediamine tetraacetic acid (EDTA), gallic acid, quercetin all from Sigma Chemicals Co. (St. Louis, MO, USA).

Statistical Analysis

Statistical analysis was performed by Graph Pad Prism 6 statistical software. Results are expressed as mean ± SD and analyzed by ANOVA and Tukey tests with univariate rate determination of significance with $P \leq 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Contents of total phenols and flavonoids bark extracts of *Myrianthus holstii*

The levels of total phenols and total flavonoids of bark extracts of *Myrianthus holstii* are determined from the calibration line y = 0.004 x + 00; R^2 = 0.998 and y = 0.037 x + 00; R^2 = 0.997 plotted using standard as gallic acid and quercetin, respectively.

Table-1: Levels of total phenols and total flavonoids of bark extracts of *Myrianthus holstii* (Mean ± SD of three trials)

Extracts	total phenols mg GAE / g of extract	total flavonoids mg QE / g of extract
EAec	27.67 ± 1.45	12 ± 0.57
EEec	428.70 ± 1.67	53.33 ± 3.75

Medium extracts the same column with different letters (a, b) with superscript are significantly different from the smallest to the largest average at $P \leq 0.05$.

Total Phenol and Flavonoid Content

The total phenol content of M. holstii extracts was calculated from the calibration curve using gallic acid as the reference polyphenol. The results obtained are expressed in milligram gallic acid equivalent per gram of dry matter (mg EAG / g extract).

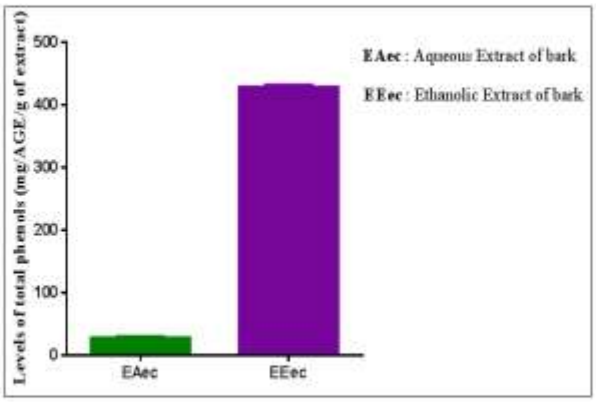
The Folin-ciocalteu method from the equation of the regression line (y = 0.004 + 00; r2 = 0.998) of the calibration range of gallic acid (0-0.5mg / mL), shows the content of the ethanol extract of the bark (EEec) of M. holstii with a value of 428.70 ± 1.67 mg EAG / g of extract is higher than the aqueous extract of bark (EAec) which has a value of 27, 67 ± 1.45 mg EAG / g dry matter.

This value is greater than that found by [21], who worked on extracts of the root bark of another genus of the plant named Myrianthus arboreus. He reports that the ethanolic extract gives a total phenolics content of 292 ± 1.3 mg EAG / g extract. This difference in levels can be explained by environmental conditions, climatic and collection period and also by genetic factors and experimental conditions.

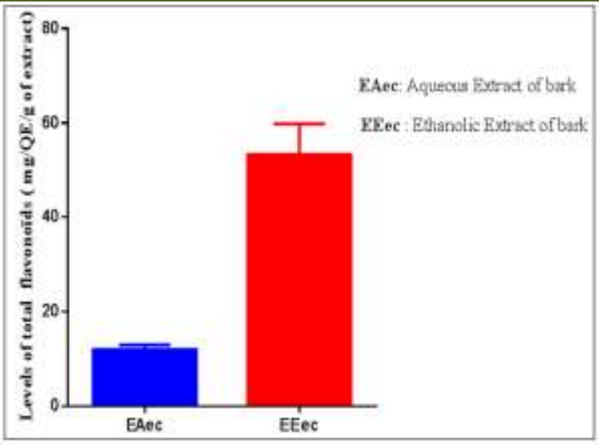
As for the flavonoids, the assay was performed by the colorimetric method of aluminum trichloride AlCl3. The flavonoid content of the different parts of the plant is expressed in milligram equivalent of quercetin per gram of dry matter (mg EQ / g MS) as shown (Graph 2). Quercetin, considered to be a positive control, made it possible to produce a calibration curve obtained with the straight line regression (y = 0.037 + 00, r2 = 0.998), established with the standard standard of quercetin (0-100 mg / mL).

The results of the quantitative flavonoid assay show that the ethanolic bark extract (EEEC) is higher in flavonoids with 53.33 ± 3.75 mg EQ / g extract than the aqueous extract bark with a value of 12 ± 0.00 mg EQ / g extract. It is found that the EA of M. holstii bark has significantly higher flavonoid content than the ethanolic extracts of the root bark of the Myrianthus arboreus plant which has a value of 3.6 ± 1.3. These can be explained by the difference in ecological conditions, such as soil type, microclimatic conditions, geographical position, site, age and vegetative stage of plants and fruits [22].

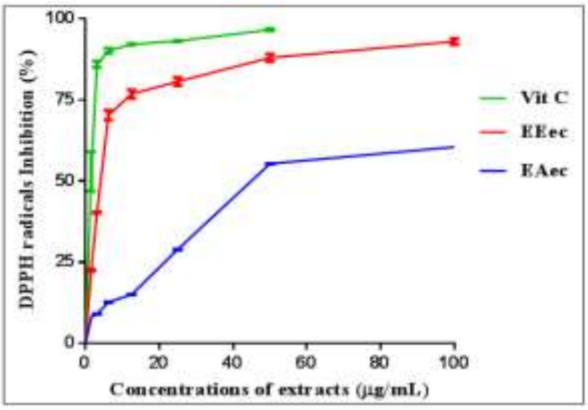
EE 70% Myrianthus holstii represent the highest levels of total phenols and flavonoids. This result confirms the great richness of barks in phenolic compounds. The results obtained confirm that the different extraction solvents used show differences in their ability to extract the phenolic compounds from M. holstii. According to [23], the combined use of water and organic solvent can facilitate the extraction of chemicals that are soluble in water and / or in the organic solvent. Thus, the polarity of the solvent plays a key role in increasing the solubility of phenolic compounds [24].



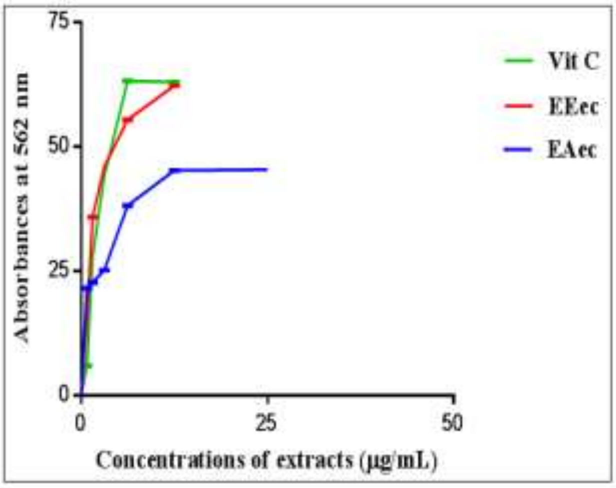
Graph-1: Levels of total Phenols of Aqueous and Ethanolic Extracts of *Mynathus Holstii* Bark (mg/AGE/g of extract) (Mean ± SD of tree trial)



Graph-2: Levels of total Flavonoids of Aqueous and Ethanolic Extracts of *Mynathus Holstii* Bark (mg/QE/g of extract) Mean ± SD of tree trial)



Graph-3: Evolution of the Antiradical of the Aqueous and Ethanolic Extracts of *Myrianthus Holstii* Bark



Graph-4: Chelating Power of Aqeous Extracts and Ethanolic of *Myrianthus Holstii* Bark

Table-2: Antiradical and chelating power of *Myrianthus holstii* bark extracts and vitamin C resulting in 50% reduction of the DPPH radicals and ferrous ion chelation

Extracts and compounds	Antiradical activity CI_{50} (µg/mL)	Chelating powder CI_{50} (µg/mL)
EAce	$43,30 \pm 0,40^{b}$	Nd^{a}
EEec	$03,20 \pm 0,45^{a}$	$04,25 \pm 0.17^{a}$
Vit C	$01,25 \pm 0.02^{a}$	$04,08 \pm 0,01^{a}$

For antioxidant activity, antioxidants reduce and decolorize the DPPH radical, to a yellow compound diphenyl picryl hydrazine, the extent of the reaction will depend on the ability of antioxidants to give the hydrogen [25]. The results are expressed as a percentage of the antiradical activity and expressed using the IC50 parameter, which is defined as the concentration of the substrate that causes a 50% loss of DPPH activity [26]. The measurements of the radical power of M. holstii reveal that the EEEC is more active with an IC50 of the order of 03.20 ± 0.20 µg / mL and the EAec has the lowest anti-radical activity with an IC 50 = 43.30 ± 0.40 µg / mL (Figure 3). Vitamin C used as a reference molecule has an IC50 of the order of 01.25 ± 0.02 µg / mL close to the EEEC.

Moreover, at 100 µg / mL, EAec and EEec extracts respectively have 60% and 93% inhibition of the DPPH radical.

Previous studies have confirmed that phenolic compounds are the main antioxidant constituents in medicinal plants, vegetables, fruits and spices. Phenolic compounds can exhibit strong antioxidant activity in vitro, they directly trap reactive oxygen species [27]. According to [28] polyphenols appear to be efficient donors of hydrogen to the DPPH radical, because of their ideal structural chemistry [28] found that black tea extracts with very high antioxidant activity also have a higher polyphenol content. The other minor phenolic compounds should not be neglected, so that the synergy between the different chemicals with each other should be taken into account in the biological activity [29].

On the other hand, the antioxidants in a mixture make the antioxidant activity not only dependent on the concentration, but also on the structure and nature of the antioxidants [30]. The chelating activity of the aqueous and ethanolic extracts

of M. holstii bark revealed that the EEEC with an IC 50 = 0.45 ± 0.17 µg / mL is the most active and the EAEC less active, which does not not revealed significant chelating activities (Figure). Moreover, vitamin C used as a standard molecule with an IC50 of 04.08 ± 0.01 µg / mL close to our EEec.

The value of IC50 obtained with vitamin C is very low (04.08 ± 0.01 µg / mL), reflecting its strong chelating effect, this value is very close to the IC50 of 5.6 µg / mL reported by [16]. In a study conducted on extracts of fourteen barley varieties, [31] highlighted the existing of a very low and insignificant correlation ($r = 0.041$, $p \leq 0.05$) between the chelating activity of these extracts and their contents of phenolic compounds.

In addition, the chelating capacity of a phenolic compound is dependent on the availability of a number of suitably oriented functional groups [32]. So a sample rich in phenolic compounds could not chelate the transition metals if its polyphenols do not have the functional groups necessary for the chelating activity.

CONCLUSION

The present work allowed us to study the antioxidant activity and to determine the total phenol and flavonoid contents in the aqueous and ethanolic extracts 70% of the bark of M. holstii, a local plant which represents a source of natural substances bioactive. On the basis of the results obtained, the determination of the phenolic compounds contained in the two extracts revealed a high content in the ethanolic extract. Similarly, the antioxidant activity of the ethanolic extract was greater than the aqueous extract and even close to vitamin C.

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